

attachment affect the efficiency of force transfer onto the filament, which in turn results in fluctuations of the gliding velocity as shown theoretically by Sekimoto and Tawada [1]. The theory establishes a relationship between the motional diffusion coefficient, the gliding velocity, the motor density, and a factor capturing the angular variations in the motor attachment. We present measurements of the motional diffusion coefficient for microtubules gliding on full-length kinesin-1 with known surface density. Measurements at varying ATP concentrations and kinesin surface densities enable, for the first time, measurements of the geometry factor. The implications of these measurements for the underlying motor attachment geometry as well as the efficiency of force generation by coupled motors will be discussed.

1. Sekimoto, K.; Tawada, K. *Phys Rev Lett* 1995, 75, (1), 180-183.

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Novel Optical Trapping Methodology Reveals Cooperative Function of Kinesin Motors on Cellular Organelles

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Optical trap force measurements give a precise readout of how motor proteins function against a calibrated load. To understand how motors work inside cells, optical trapping must be extended to motors transporting real cellular organelles. However, the size of organelles in a cell is variable and unknown. To date, it has not been possible to precisely calculate the force exerted by an optical trap on single cellular organelles.

We have reconstituted robust kinesin-driven in vitro motility of lipid droplets in a cell extract of mammalian liver. A new method of optical trapping is then used to calibrate the optical trap precisely for every single lipid droplet, irrespective of its size. We have measured and analyzed the cooperative function of multiple endogenously assembled kinesins against a calibrated load-force with precision and spatio-temporal resolution comparable to kinesin-coated beads.

Our method of force measurement makes accessible for experimental scrutiny a whole new range of physiologically important questions: How much force do motors exert on cellular cargoes? How are motors assembled on the cargo membrane? What is the emergent function of such motor-assemblies? Do multiple motors compete or cooperate? How do motor-associated regulatory proteins fine tune this process? How does emergent function of kinesins differ from dyneins? Our work will answer some of these questions.

Finally, our method of force measurement provides for the first time an ability to biophysically interrogate how motors may regulate storage and lipolysis of cytosolic fat in mammals. This may further our understanding of diseases related to (mis)management of fat.

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Nanometer Precision in Filament Localization allows for Precise Off-Axis Tracking of Molecular Motors

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Measuring the exact path of molecular motors, such as cytoskeletal motor proteins, on their tracks has proven to be difficult without knowing the precise location of the filaments. Up to now, off-axis stepping has therefore mostly been inferred from the tracked positions of the motors with respect to the fitted path of the motors instead of determining the filament centerline. Obviously, this limits the precision of the measurements and may lead to errors due to the sometimes complex three-dimensional structure of the filaments. We developed a filament tracking algorithm to determine the centerline position of fluorescently labeled filaments with nanometer precision. This allowed us to observe the non-parallel movement of kinesin-1 motors with respect to the microtubule centerline, which is consistent with kinesin-1 following a protofilament of a supertwisted microtubule. Combined with methods to measure nanometer heights above substrate surfaces, such as fluorescence interference contrast or parallax, our algorithm presents a promising tool for optical 3D-nanometry.

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Effect of Charge on Autoinhibition of Kinesin-1

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Kinesin-1 is autoinhibited at physiological salt concentrations through an inhibitory interaction of C-terminal tail domains with the N-terminal motor (head) domains. This interaction of heads with the tail is highly dependent on the ionic strength, with high ionic strength strongly favoring dissociation. Unexpectedly

the binding stoichiometry is one tail peptide binding to the two heads of a motor domain dimer, as originally indicated by biochemical titrations and recently confirmed by the determination of the structure of a head dimer - tail complex by X-ray crystallography (Kaan, et al., *Science* 333, 832 (2011)). A short region of the tail centered on lysine-944 (the IAK region) is sufficient for binding of a single tail to two heads. However, the binding affinity of this short region on its own is low. Tight binding of the tail to the heads requires the inclusion of an adjacent region with a large excess of positive charge. This positive region is designated the auxiliary binding site (ABS) because it is also responsible for the nucleotide-independent binding to the tail to microtubules. Kinesin motor domains have several negatively charged side chains near where the heads are cross linked by the tail domain. Mutation of these negative head residues to Ala decreases the affinity for heads of tail domains that contain both the ABS and the IAK region. This suggests that the increased affinity produced by the ABS region is due to interactions with these negative regions of the heads. Supported by NIH grant NS058848.

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Biochemical Characterization of the Rice Kinesin O12 and N14 Belonging to Kinesin-14 Family

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Kinesin is an ATP driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesins derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. Recently, the genome sequences of rice were completed. Bioinformatics analyses revealed that at least 41 kinesin-related proteins were encoded on the rice genome. In this study, we focused on the two rice kinesins; O12 and N14 that belong to kinesin-14 family. Previously, we have expressed the novel rice kinesin O12 by *E.coli*. Biochemical studies of the O12 motor domain demonstrated that O12 has very unique properties, which may reflect the plant specific physiological role. We have also succeeded to express another rice specific kinesins N14. O12 is the C-terminal motor domain. On the other hand, the motor domains of N14 are found at the central region in the primary structure. The biochemical characterizations of the kinesin N14 motor were studied. ATPase activities and interaction with fluorescent ATP analogues, NBD-ATP were analyzed and compared with other rice kinesins and conventional mouse kinesin. Interestingly, microtubule-dependent steady state ATPase activity of N14 motor domain was extremely lower than that of other kinesins. Kinetic analyses using stopped-flow also demonstrated that ATP binding to N14 in the presence of microtubule was extremely slow in comparison with other kinesins. It is suggested that the rice kinesin N14 may have unique enzymatic properties that are obviously different from O12.

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A Universal Pathway for Kinesin Stepping

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Kinesin-1 is a processive motor that transports cargo along microtubules by hydrolyzing ATP in a tightly regulated stepping cycle. Efficient gating mechanisms ensure that the sequence of kinetic events proceeds in proper order, generating a large number of successive reaction cycles. To study gating, we created two mutant constructs with extended neck linkers and measured their properties using both single-molecule optical trapping and ensemble fluorescence techniques. Due to a reduction in the inter-head tension, the constructs can access an otherwise rarely populated conformational state where both motor heads remain bound to the microtubule. ATP-dependent, processive backstepping and futile hydrolysis were observed under moderate hindering loads. Based on measurements, we formulated a comprehensive model for kinesin motion that incorporates reaction pathways for both forward and backward stepping. In addition to intramolecular strain, we find that neck-linker orientation is also responsible for ensuring gating in kinesin-1.

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In Vitro Study of the Direct Effect of Microtubule Acetylation and Detyrosination on Kinesin Motility

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Kinesin-1 is a processive, plus end-directed motor that is a major component of microtubule-based intracellular transport. It has been previously shown through in vivo studies that kinesin-1 preferentially translocates along certain subsets of